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The Patent Office

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference

C1422/P

 Patent application number (The Patent Office will fill in this part)

0224067.9

PerkinElmer UK Limited 204 Cambridge Science Park Milton Road Cambridge CB4 OGZ

Patents ADP number (if you know it)

each applicant (underline all surnames)

3. Full name, address and postcode of the or of

If the applicant is a corporate body, give the country/state of its incorporation

8485385001

1

4. Title of the invention

Improvements in and relating to imaging

5. Name of your agent (if you bave one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Keith W Nash & Co

90-92 Regent Street Cambridge CB2 1DP

Patents ADP number (if you know it)

1206001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number (if you know it)

Date of filing
(day / month / year)

 If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' 15:

Yes a)

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.See note (d))

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C1422/P

Title: Improvements in and relating to imaging

Field of invention

This invention concerns a method and apparatus by which fluorescing elements of a

specimen or sample can be imaged for analysis.

Background

It is known to use laser excitation confocal imaging, a Nipkow disc scanner (which may be a microlens enhanced disc scanner) and a digital camera, to provide image data in two dimensions, and if controlled Z-axis movement is also provided, three dimensional image data can be obtained. Where a fluorescing element is altering with time, a sequence of

images can be obtained to provide data in four dimensions, (ie area, depth and time).

One system which is capable of providing such data about microscopic specimens such as cells and tissue samples is the UltraVIEW live Cell Imager as produced and supplied by Perkin Elmer Life Sciences. This system uses a confocal laser microscope and CCD camera imaging system such as is described in a paper written by Kawamura Negishi Otsuki and Tomosada entitled Confocal Laser Microscope and CCD Camera, published in

the Yokogawa Technical Report No. 33 (2002), English Edition.

It is an object of the present invention to provide an imaging system which is an improvement over the current UltraVIEW system and that described in the aforementioned paper.

It is also an object of the present invention to provide a control system for more accurately controlling the imaging and data capture process and a method of operation of such a system.

It is also an object of the present invention to provide an improved method of capturing light from fluorescing elements in a sample or specimen and converting the captured light into data for processing for analysis and/or display and/or storage for subsequent display and/or processing for analysis.

Summary of the invention

According to one aspect of the present invention in a method of imaging light from a specimen in which excitation light passes to the specimen through openings in a rotating Nipkow disc, light emitted by fluorescence of the specimen passes in the opposite direction through the disc to form an image for capture by an image capture device such as a CCD camera, and in which rotation of the disc through an angle of A° results in the scanning of the whole of an area of interest of the specimen by the openings in the disc, the excitation light and/or the image capture device are controlled so that light emitting from the specimen is only incident on the capture device for a time period equal to that required to rotate the disc through nA° where n is a whole number equal to or greater than 1.

The area of interest may be the whole of the area of the specimen or a part thereof.

A single Nipkow disc may be employed, but more preferably a 2-disc arrangement is employed, one containing openings and the other microlenses, as described in the Yokogawa Technical Report. References herein to a disc (or disc means or disc arrangement) are intended to include multiple disc assemblies such as described in that Report, as well as single disc arrangements, where the content so admits.

In a preferred method, light is prevented from reaching the specimen and the camera except from the excitation source, the image capture device is a CCD camera, the camera

chip is read out at the end of each said time period and the charge thereon is replenished prior to the beginning of the next said time period, and the source of excitation light is switched on for a time precisely equal to the said time period and thereafter is extinguished, so that the duration of the camera exposure is controlled by the time for which the excitation light source is active.

Alternatively or additionally, shutter means may be provided to interrupt light from the excitation source except for when the specimen is to be scanned, and the excitation light source may be operated continuously or for longer periods of time than the said time periods.

Shutter means may be provided between the Nipkow disc and the camera to prevent light reaching the camera except for the specific periods of time during which the excitation light is incident on the specimen, thereby to reduce errors which could arise from light arising from phosphorescence, afterglow, stray reflections or other effects, from reaching the camera.

If shutter means is provided for both source and camera, they are preferably synchronously operated such as by mechanical or electrical means or both.

Where the specimen is largely transparent it can be scanned in different parallel spaced apart planes and to this end, where the method also involves movement of the specimen or the Nipkow disc arrangement along the so-called Z axis (ie along the optical axis) so that the incident light can be brought to a focus at different points along that axis to enable the specimen to be scanned in different parallel spaced apart planes, the movement is preferably restricted to periods during which at least excitation light is inhibited (or prevented from reaching the specimen) and preferably is restricted to periods during which the camera is rendered insensitive to light.

Typically the Z-axis movement is performed at the end of each said time period corresponding to nA° of disc rotation (or after N successive such time periods), and may be such as to increase or decrease the distance between disc and specimen.

The Z-axis movement is preferably made in discrete steps each of a predetermined equal size.

Each Z-axis movement between exposures may be the same or a different distance (typically made up of one or a whole number of said steps).

Where the time required to alter the Z-axis spacing is greater than the minimum time needed between exposures, the method preferably includes the step of adjusting the time between exposures or controlling the illumination and camera exposure by reference to the Z-axis movement, such that excitation illumination and exposure is only initiated after a sufficient period of time has elapsed or after a desired Z-axis movement has been achieved.

Where the method involves variation of the wavelength of the illuminating light from one exposure to another, the method preferably also includes the step of altering the illumination wavelength between the end of one exposure and the beginning of the next.

Where the variation of excitation light wavelength is to be effected whilst focussing the light onto the same plane of the specimen (ie there is no Z-axis movement between each of a succession of two or more illuminating and exposure steps making up a sequence of exposures), albeit each employing to a different wavelength of excitation light, the Z-axis movement is initiated only after each complete sequence of exposures has been performed, and the next sequence of such steps is only initiated after each Z-axis movement has been completed.

Wavelength variation may be achieved by employing two or more light sources of different wavelengths and selectively operating the sources or selectively directing light from the sources in turn, as required, to the Nipkow disc and specimen.

According to a preferred feature of the invention the excitation light may be obtained from one or more laser light source.

In a preferred arrangement, a single light source is employed which comprises an accoustic optically tuned filter (AOTF) crystal and the wavelength of the emitted light is altered by altering the frequency controlling signal to the crystal as required. Such an arrangement may contain a single laser source, or two or more laser light sources whose outputs can be optically combined.

Preferably the alteration of the frequency is effected between exposures.

Alternatively a luminescent light source such as a monochromator or white light source fitted with controllable filters may be used.

Where the intensity of the excitation light incident on the section is to be adjusted from one exposure to another, the method preferably involves the step of adjusting the intensity of the incident illumination between exposures. This may for example be achieved by interposing neutral density filters, or opening or closing an iris diaphragm in the light path, or adjusting the power to the light source, or any combination thereof.

Where the intensity of the illumination at one wavelength is different from that at another due to inherent intensity variation as between one source and another, or between different modes of operation of a single crystal, the intensity of the illumination between one wavelength and another may be adjusted so as to provide a predetermined intensity of illumination at the specimen for each wavelength.

Typically but not exclusively, the power adjustment from one exposure to another as wavelength varies, is such as to ensure a substantially similar level (ie intensity) of illumination at the specimen during the scanning making up each exposure, independent of wavelength.

A similar technique may be employed if the emission from the sample differs according to wavelength of the fluorescence emitted, and in order to render the emitted light of similar intensity (irrespective of wavelength), the power adjustment from one exposure to another may also, or instead, be adjusted to provide substantially constant intensity fluorescence irrespective of the wavelength thereof.

Preferably the method is controlled from a single control centre which typically includes a programmable computer and one or more interfaces for converting signals produced by the computer into signals suitable for driving, or controlling, the supply of power to drives or devices, to effect the movements and rotations required by the different steps of the method.

Where the image capture device is a CCD camera, it is preferably cooled to increase the S/N ratio of the camera output signals.

Preferably the CCD camera is a cooled digital camera.

Since the light presented to the camera is capable of forming an image, the camera may be replaced by an eyepiece which will allow the image to be seen by the human eye, or the apparatus may include a lens which may be adapted to project the image onto a screen for viewing.

The light path to the camera may include a beam-splitting or beam directing device by which light can be split between (or divided between) the camera and eyepiece or projection system.

Two or more cameras may be employed and one or more of the cameras may be replaced by sensors.

Where two or more cameras and/or sensors are provided (with or without any eyepiece and/or projection system, the light path thereto may include a beam splitting means or beam directing means as appropriate.

The camera and/or sensor output(s) may be stored for subsequent analysis of the signals therefrom by a computer or display for visual analysis of an image produced by reading out the stored signals.

Alternatively the method may provide that the signals from the camera(s) and/or sensor(s) are processed in real time as they arise from the camera.

According to another aspect of the present invention apparatus for performing a method of imaging light emitted from a specimen, typically due to fluorescence of different parts of the specimen comprises means for mounting the specimen, a light source for producing excitation light, a Nipkow disc assembly adapted to rotate and in so doing scan the specimen with the excitation light which passes to the specimen through openings in the disc assembly, the latter being adapted to convey light emitted as fluorescence from the specimen in the opposite direction through the disc assembly, a CCD camera on which the light emitted from the specimen is focussed after passing through the disc, and in which rotation of the disc through an angle of A° results in the scanning of the whole of an area of interest of the specimen by the openings in the disc, and control means adapted to control the excitation light and/or the camera so that light from the specimen is only incident on the camera for a time period equal to that required to rotate the disc through nA° where n is a whole number equal to or greater than 1.

Preferably means is provided to prevent light from reaching the specimen except from the excitation source, and from reaching the camera except from the specimen via the Nipkow disc assembly.

Preferably the control means also controls the operation of the CCD camera so that the camera chip is read out at the end of each said time period and the charge thereon is replenished prior to the beginning of the next said time period, the source of excitation light is switched on for a time precisely equal to the said time period and thereafter is extinguished, so that the duration of the camera exposure is controlled by the time for which the excitation light source is active.

Alternatively or additionally, source shutter means may be provided which is operated by signals from the control means to interrupt light from the excitation source except for when the specimen is to be illuminated.

Alternatively or additionally, camera shutter means may be provided between the Nipkow disc and the camera, this camera shutter means being operated by signals from the control means to prevent light reaching the camera except for the specific periods of time during which excitation light is incident on the specimen, thereby to reduce errors which could arise from phosphorescence, afterglow, stray reflections or light from other effects, reaching the camera.

If shutter means is provided for both source and camera, they are preferably synchronously operated such as by mechanical or electrical means or both.

Where the specimen is largely transparent, the apparatus may also include drive means adapted to move the specimen or the Nipkow disc arrangement along the so-called Z axis (ie along the optical axis) so that the incident light can be brought to a focus at different points along that axis, to enable the specimen to be scanned in different parallel spaced part planes.

Preferably the Z-axis drive means is also controlled by signals from the control means.

Preferably Z-axis movement is restricted to periods during which at least excitation light is inhibited (or prevented from reaching the specimen) and is preferably restricted to periods during which the camera is rendered insensitive to light.

Typically the control means controls the Z-axis drive so as to effect the Z-axis movement at the end of each said time period corresponding to nA° of disc rotation (or after a succession of N such time periods), and may be such as to increase or decrease the distance between disc and specimen.

Preferably the Z-axis drive is adapted to move in discrete steps each of a predetermined equal size.

The control means may control each Z-axis movement between exposures (or successions of exposure) may be the same or a different distance (typically each movement being made up of one or a whole number of equal steps).

Where the time required to alter the Z-axis spacing is greater than the minimum time needed between exposures, control means is adapted to control the time between exposures or to control the illumination and camera exposure by reference to the Z-axis movement such that excitation illumination and exposure is only initiated after a sufficient period of time has elapsed, or after desired Z-axis movement has been achieved.

Where the method involves variation of the wavelength of the illuminating light from one exposure to another, the apparatus may comprise two or more excitation light sources producing light of different wavelengths, or a single source which is adjustable to produce light of different wavelengths and the control means is adapted to select or control the source to alter the excitation light wavelength between the end of one exposure and the beginning of the next.

Where the variation of illumination wavelength is to be effected whilst focussing the light onto the same plane of the specimen (ie there is to be no Z-axis movement between each of

a succession of two or more exposure steps making up a sequence of said steps, albeit each corresponding to a different wavelength of excitation light) the control means may be adapted to control the Z-axis drive means so that Z-axis movement is initiated only after each complete sequence of said steps has been performed, and the next sequence of said steps is only initiated after each Z-axis movement has been completed.

Preferably a single excitation light source is employed, whose emitted wavelength can be altered, and preferably a laser light source is employed which comprises an acoustic optically tuned filter (AOTF) crystal and the control means is adapted to provide signals to control the wavelength of the emitted light by altering the frequency controlling signal to the crystal, between exposures.

Where the intensity of the excitation illumination incident on the specimen is to be adjusted from one exposure to another, the control means may be adapted to adjust the intensity of the incident excitation illumination between exposures.

This variation may for example be achieved by interposing different neutral density filters in the light path.

Therefore apparatus may comprise a plurality of neutral density filters and means for selectively positioning one or more of the filters in the light path.

Alternatively the apparatus may comprise an adjustable iris diaphragm in the light path and drive means for opening or closing the iris as required.

Alternatively and preferably the excitation light intensity may be controlled by adjusting the power to the excitation light source and the control means may be adapted to generate signals for controlling the light source as appropriate.

The excitation light intensity may be controlled by using two or more such intensity varying techniques in combination, typically in series.

Where the intensity of the illumination at one wavelength is different from that at another due to inherent intensity variation as between one source and another or between different modes of operation of a single source such as an AOTF crystal laser, the control means may be adapted to alter the intensity of the illumination between one wavelength and another so as to provide a predetermined intensity of illumination at the specimen for each wavelength.

Typically but not exclusively, the control means is adapted to control the power to the excitation light source from one exposure to another as wavelength varies, so as to ensure a substantially similar level of excitation illumination (intensity) at the specimen during each exposure, which level is independent of wavelength.

Preferably the CCD camera is a cooled digital camera.

Since the light presented to the camera is capable of forming an image, the camera may be replaced by an eyepiece which will allow the image to be seen by the human eye, or by a lens adapted to project an image onto a screen for viewing.

Alternatively the light path to the camera may include a beam-splitting or beam directing device by which light can be split between (or diverted to one or the other of) the camera and eyepiece or projection system.

The apparatus may include a data storage means adapted to store signals from the camera for subsequent analysis of the picture signals by a computer, or for display on a screen for visual analysis of an image produced by reading out the stored signals.

Alternatively a signal path from the camera to a computer and/or display is provided so that the picture signals from the camera may be processed for analysis and/or displayed in real time, as they arise.

It is to be understood that in place of a laser and AOTF, or a shutter, to control excitation light a diode laser source may be employed which has beam control built in. In this context reference to a shutter can be considered to refer to switching or modulating a diode laser source.

The invention will now be described by way of example with reference to the accompanying drawings in which:

Figure 1 is a schematic diagram of a Nipkow Disc Confocal microscope system;

Figure 2 is a system diagram giving details of proprietary hardware which can be connected to the computer based controller of Figure 1;

Figure 3 is a schematic diagram of the control system for controlling an AOTF laser;

Figure 4 is a schematic diagram showing the different machine states, and

Figure 5 is a block schematic diagram of the controller board.

Reference is made to Figures 1 to 3 and the related description of the Yokogawa Technical Report No. 33 (2002) for a description of the operation of the CSU10 system.

Figure 1 shows a Yokogawa confocal microscope 10 (which may be a model CSU 10 or the more recent model CSU21), set to view a sample (not shown) on a stage 12 and provide light to a camera 14 to form an image thereon of fluorescence arising from excitation of the sample by laser light from an AOTF laser source 16.

A controller 18 converts signals from a PC 20 into control signals for operating or controlling the operation of items 10, 12, 14 and 16 and in turn can transmit data from these items to the PC 20, if required. Software 22 is shown loaded on the PC enabling the latter to be instructed to perform particular tasks or control items 10 to 16 in different

ways, depending on the sample or experiment to be performed. Alternatively different software can be loaded as required to enable the system to perform different tasks or experiments.

Figure 2 shows the various items of proprietary hardware which can be interconnected to constitute an operating example of the system of Figure 1. Essentially the controller 18 of Figure 1 is labelled as the synchronisation count 18 in Figure 2 and the PC 20 is identified as a Dell Dimension GX260 loaded with Windows 2000 as the basic operating system.

The microscope 12 is typically a Nikon eclipse TE300 and the confocal scanning unit a Yokogawa CSU10 or CSU21 unit.

Z-axis adjustment is achieved using a Physik Instrument piezo electric driver 22 which is controlled by a proprietary control unit 24 provided with analogue control signals from 18.

The laser excitation light source is an Omnichrome Series 43 (3 wavelength) laser as supplied by Meles Griot Laser Group of California operating with an AOTF crystal and controller from NEOS of Florida. The light intensity of the laser is controllable from the Control band 18" which with the synchronisation unit 18' makes up the controller 18 of Figure 1. A driver 26 controls the AOTF control 28 for the laser 16 whose power supply is shown at 30.

The camera 14 is a Hammatsu Orca EP CCD camera.

A power supply for the driver 26 is shown at 32 and a power supply and interface for the camera 14 is shown at 34.

The synchronisation circuit 18' is connected to the PC 20 via the USB connection 36.

Figure 3 shows details of the AOTF laser, as comprising an Argon ion laser 38 and auxiliary laser 40. An AOTF crystal 42 controls the wavelength of the light emitted via fibre 44 and the CSU10 fibre port.

The crystal 42 is controlled by an RF signal from the controller 46 which in turn is controlled by a TTL analogue signal from the computer via circuit 18' and driver 26 (see Figure 2).

The State diagram of Figure 4 shows the 1 KHz clock signal controlling the different activities required within the system.

Figure 4 represents a state machine consisting of a set of output registers labelled line on/off (48), laser line (50), Z position (52), camera (54), and head sync (56), a duration down counter (58), a state counter (60), and a list of next states comprising a set of bit patterns in a memory (62). Some of the bit outputs of the output registers are connected to digital ports on devices which can be controlled by such signals, such as the laser line selected on the AOTF systems, the camera trigger and the CSU scanning disc system synch input. Values from output registers 50 and 52 are converted from digital to analog signals by DAC's 64, 66 to provide analog signals to analog ports on the AOTF system and the 2-axis piezo drive.

A clock signal, typically of 1KHz, is supplied to the duration downcounter 58.

The state data may be loaded into the memory 62 from the host computer.

At initialisation, the state counter 60 is loaded with the number of states. The first state is transferred from the first line in memory 62 to the output registers 48 to 56, where it sets the states on the devices controlled thereby. The intended duration of the first state is loaded into the duration down counter 58 from state duration memory 68.

At the next clock pulse, the duration counter 58 is decremented by one and its count value compared with zero. If non-zero, the output registers are held until the next clock pulse.

When the duration counter 58 reaches zero, the state counter 60 is clocked and points to the next state in the memory. This next state is transferred from the memory to the output registers at 56, where it sets the states on the devices controlled thereby and the duration for this next state is transferred from 68 to the duration counter 58 to establish the duration of this next state.

The system operation progresses until the state counter 60 is decremented to zero at which point the operation stops.

The system may be set up to start again upon this stop signal.

Messages relevant to different operations/states are set out below.

USB Message Types

Interrogation

This message is a work around to cope with initial lack of a USB vendor ID. The USB master interrogates each unit to find out what type it is.

Message type	0x00	1 byte
Filler (usually all 0x00)		3 bytes
		4 bytes

The synchroniser replies with:

Message type	0x00	1 byte
Second byte of interrogation message echoed (i.e. 0x00)		1 byte
0x12 (i.e. ASCII DC2)		1 byte
0x36 (i.e. ASCII '6')		1 byte
		4 bytes

Initialisation

This message is sent before any experiments are conducted, to set the default output state and the confocal head parameters.

Message type	I	1 byte
Digital outputs (Camera trigger bit 7, bits 0 to 6 general purpose)		1 byte
AOTF bits		1 byte
Z stepper position (see note 1)		2 bytes
Sync pulse on period, ms (see note 2)		1 byte
Sync pulse off period, ms (see note 2)		1 byte
Sector time, ms (see note 2)		1 byte
		8 bytes

Z-axis drive

Z-axis stepper motor position is given by:

 $Pz/(100\mu m/2^{16})$

Or

Pz/(1.526nm)

Where Pz is the desired Z stepper position; assuming a sensitivity of $10\mu\text{m}/\text{volt}$ and a 10 volt output span. Up to 16 bits are available for this value, but the embodiment being described responds only to the most significant 12 bits, which gives a resolution of 25nm.

Sector time sychronisation

Sync pulse times, number of sectors and camera exposure times are all constrained by the requirement for every event to be synchronised to sector times.

For example, a 12 sector disk could have the following parameters.

Disk RPM 5000 2500 1666.66 7 Disk Hz 83.3333 41.6666 27.7777 3 7 8

The Yokogawa CSU10 and CSU21 systems would be limited to 3ms per sector in the present design.

State data header and footer

The following message is sent before (header) and after (footer) the list of State Data. Any State Data that is already stored is cleared when the header is received. The message is ignored if the synchroniser is running. The messages are referred to as State list headers and State list footers.

Message type	H	1 byte
Sub-type		1 byte
H: Header		
• F: Footer		
		2 bytes

State Data

State data should immediately follow a state list header. The synchroniser is set to check that the correct number of states are received before executing anything.

Message type	D	1 byte
Time for this state to persist (in sector widths)		2 bytes
Digital outputs (Camera trigger bit 7, bits 0 to 6 general purpose)		1 byte
AOTF bits		1 byte
Z stepper position		2 bytes
		7 bytes

Experiment control

Once State Data has been downloaded, the system can be controlled to perform an experiment.

Message t	vpe	С	1 byte
Action			1 byte
code:			
L	Start live mode (immediate data is accepted and retain		
0	Start experiment to run once (immediate data is ignore		1
S	Start experiment in continuous loop (immediate data is ignored)	3	
\mathbf{C}	Stop cleanly (stop execution at the end of the state list)	
1	Stop immediately (stop everything and revert to the initialisation data)		
			2 bytes

Immediate data

If capture is started in live mode, the digital outputs, AOTF blanking and Z stepper may be altered at will. The camera trigger bit (digital output bit 7) is ignored in all immediate data.

Message typ	e N	1	1 byte
Data type:			1 byte
Č	Digital output data (1 byte follows)		
A	AOTF blanking data (1 byte follows)		
${f z}$	Z stepper position (2 bytes follow)		
Data			2 bytes
			5 bytes

AOTF/DAC control

This message is sent to set the AOTF channel power. It would not be sensible for the AOTF levels to be changed during an experiment, but for simplicity the synchroniser will always respond to them – this is so that they can be tweaked while in live mode.

Message type	A 1 byt	:e
AOTF address (1 to 8)	1 byt	:e
AOTF data	1 byt	:e
	3 byt	tes

state	line on/off	laser line	Piezo	camer	sync	count
		<u> </u>		a		
0008	0000	0000	0110	0	1	0001
0007	0010	0200	0110	0	0	0001
0006	0010	0200	0110	1	0	0002
0005	0010	0200	0110	0	0	0001
0004	0010	0000	0110	0	0	0001
0003	0001	0100	0330	0	0	0001
0002	0001	0100	0330	1	0	0001
0001	0001	0100	0330	0	0	0001
0000	0001	0100	0330	0	0	0001

State machine sample content

System carries out a given state for 'count' clock ticks

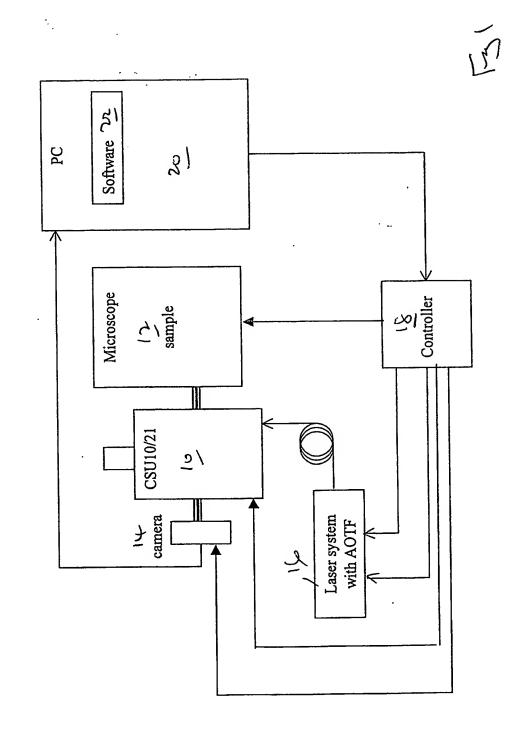
Outputs persist at given value for this time.

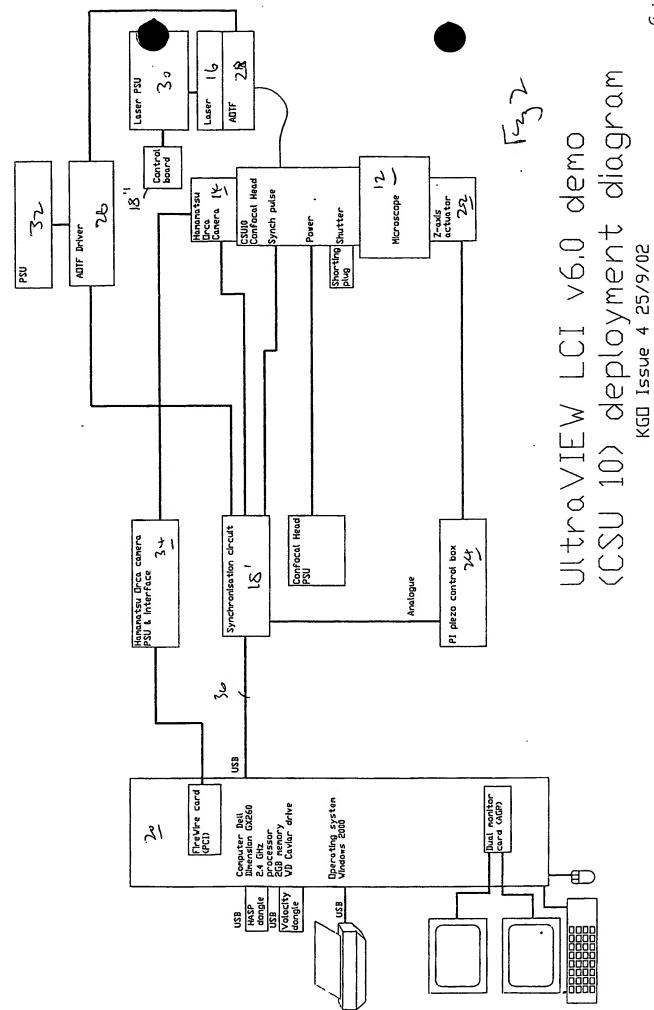
As described the invention provides a control system or synchroniser to sequence a Nipkow disc laser scanning confocal microscope imaging system having a Z-axis stepper motor drive. The system requires a host computer (20) and controller (18) and is programmable to perform a wide range of tasks and is connectable to a Nipkow disc confocal microscope hardware via a USB connector. The controller (18) may be constructed as shown in Figure 5, and based on a 8051 microcontroller which is programmed to comprise the state machine described with reference to Figure 4. The unit (18) includes a clock input 68 the DAC's 64 and 66 and output posts as follows.

- USB 70 1 port for configuration, timing and power.
- Sync output port 72 variable 0-3Vpk-pk, programmable high and low periods in 1ms increments, as described with reference to Initialisation
- TTL output port 74 8 bits for AOTF switching, up to 8 bits for camera trigger, shutter control and/or general purpose use. Synchronised to disc sector speed. One bit is dedicated to camera triggering, the rest may be controlled asynchronously when in live mode.

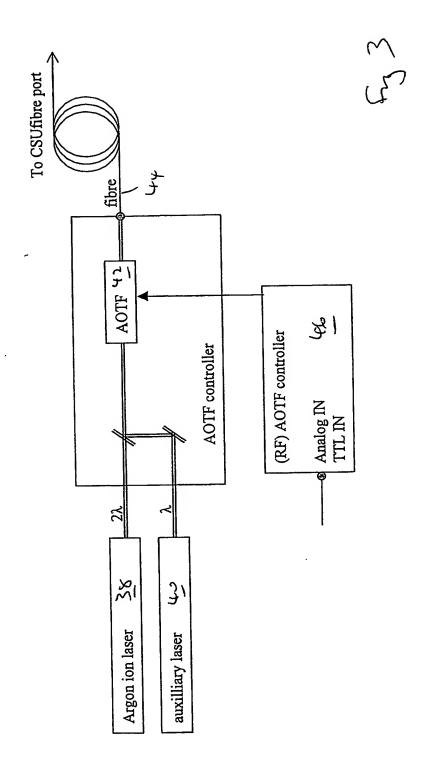
- Analogue output from DAC 64 8 channels, 0-5 volts, 8 bit resolution. For AOTF power setting and/or general use. Not synchronised, but may be controlled asynchronously.
- Analogue output from DAC 66 0-10 volts, 12 bit resolution. Synchronised to disc sector speed, or may be controlled asynchronously when in live mode.

System diagram

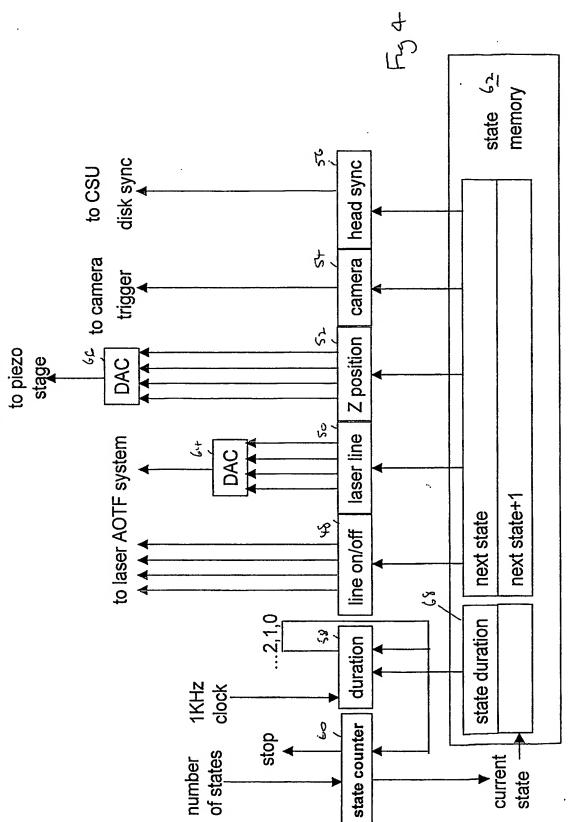




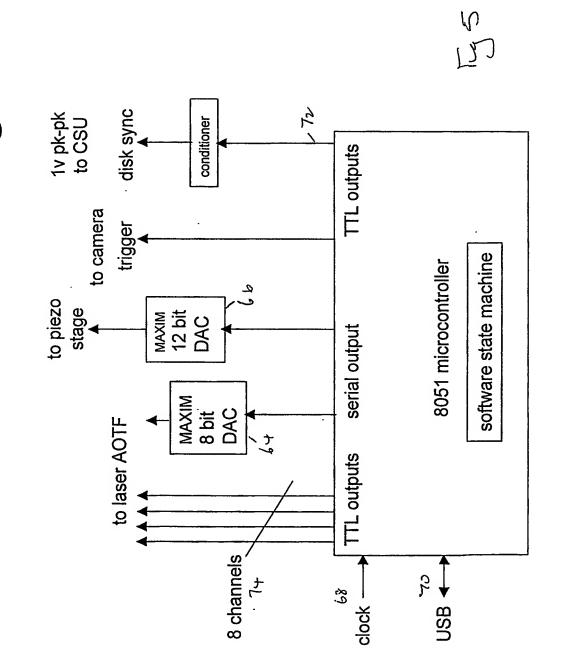
Laser AOTF control







Controller design



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